

## REMARKS

Claims 1-48 are pending in this application. Claims 1-27 and 31-48 have been withdrawn from consideration. Claim 28 has been amended to better clarify what applicants believe to be the invention. New claims 49-56 have been added for consideration. Support for the amendment and for the new claims can be found throughout the specification and in the original claims as filed, but particularly in the specification on page 5, lines 11-24; on page 13, lines 12-24 and in Figures 9A and 9B and Figure 18; page 18, lines 18-22; page 19, lines 2-4; page 23, lines 2-5; page 31, lines 10-33, continuing on to page 32, lines 1-28; page 33, lines 6-10, lines 20-27; page 34, lines 3-30; page 55, lines 23-32, continuing on to page 56, lines 1-6; page 71, lines 20-34, continuing on to page 72, line 1; page 73, lines 26-34, continuing on to page 74, lines 1-30; page 75, lines 10-26; in example 9, on pages 88-96; on page 92, lines 1-23 and particular on page 95, lines 4-32. Thus, as a result of the foregoing amendments, claims 28-30 and new claims 49-56 are under consideration.

The Examiner has objected to the specification since the specification on page 1 does not reflect the priority status of the present application. Applicants have amended the specification as suggested by the Examiner. Accordingly, withdrawal of the objection is respectfully requested.

The Examiner also alleges that no IDS has been filed in the present application and that the previously filed and signed IDS, submitted with the filing of the present application, was not considered in this application. Applicants respectfully point out to the Examiner that the MPEP states in Chapter 600, under 609 I.A.2. Continuation Applications or Divisional Applications Filed under 37 CFR 1.53(b)...that:

*“ The Examiner will consider information which has been considered by the Office in a parent application when examining (A) a continuation application filed under 37 CFR 1.53(b) or filed under former 37 CFR 1.60, (B) a divisional application filed under 37 CFR 1.53(b) or filed under former 37 CFR 1.60, or (C) a continuation-in-part application filed under 37 CFR 1.53(b). Such information need not be resubmitted in the*

*continuing application unless the applicant desires the information to be printed on the patent."*

Accordingly, Applicants request consideration of the references submitted in the IDS filed with the parent application, to which the present application claims priority.

The Examiner has rejected claims 28-30 under 35 U.S.C. 102(b) as being anticipated by Engleman et al. (WO 94/02156). The claims have been amended to better clarify what Applicants regard as the invention, and have provided arguments as to the differences between the cited reference and the present invention as currently claimed. Withdrawal of the rejection is respectfully requested.

### ***Objections to the Specification***

The Examiner has objected to the specification for the following reason: The specification on page 1 should be amended to reflect the priority status of the present application. Applicants have amended the specification as requested by the Examiner, and as such, withdrawal of the objection is respectfully requested.

### ***Information Disclosure Statement***

As noted above, the Examiner alleges that no IDS was submitted for consideration in the present application. Applicants respectfully assert that upon filing the present continuation application, the IDS that was submitted with the parent application, USSN 09/251,896, now U.S. patent 6,602,709, was also submitted for consideration in the present application. Applicants respectfully request entry of the previously filed IDS for consideration in the present application.

### ***Rejections under 35 USC 102(b)***

The Examiner has rejected claims 28-30 under 35 U.S.C. 102(b) as being anticipated by Engleman et al. (WO 94/02156). Applicants respectfully traverse the Examiner's rejection, and have amended the base claim to better clarify what Applicants believe to be the invention. Support for the amendment can be found throughout the specification, and particularly on page 71, lines 20-24; page 73, lines 0-4; page 74, lines 9-14; page 75, lines 17-26; page 91, lines 16-28; page 92, lines 1-23; page 93, lines 30-33

and page 95, lines 15-32.

The Examiner alleges that Engleman et al. teach a method of assessing cytotoxic T lymphocyte activity comprising contacting antigen presenting dendritic cells with a variety of antigen donors including bacterial, parasitic, fungal, viral, and tumor antigens. The antigens may be purified, recombinant, or exist as whole organisms or cells in viable or dead form. The reference further teaches exposing antigen presenting dendritic cells to a population of T lymphocytes to be assayed for their ability to exhibit killer cell activity and assaying the cytotoxic activity of the T lymphocytes exposed to the antigen presenting DCs. The Examiner further alleges that although the reference does not specifically teach contacting the dendritic cells with “apoptotic cells”, Engleman et al. teach that pulsing DCs includes contact with irradiated cells.

Applicants respectfully traverse the Examiner’s rejection and assert that in order for a rejection under 35 U.S.C. 102(b) to be proper, the reference(s) must recite each and every element of the invention as claimed. Applicants assert that Engleman et al. do not teach the methods of the present invention as currently claimed and that there are distinct differences between the teachings of Engleman et al. and the present application.

For example, to better clarify the invention, Applicants have amended claim 28 to recite “immature dendritic cells”, and “wherein said immature dendritic cells are characterized as being surface CD83 negative and DC-LAMP negative dendritic cells, and maintaining the CD83 cells CD83 negative during said contacting.” Furthermore, the claim has been further amended to recite “promoting maturation of the dendritic cells following internalization of the apoptotic cells by exposure to a maturation factor for a time sufficient to induce maturation of said dendritic cells, wherein said mature dendritic cells are characterized as being surface CD83 positive and DC-LAMP positive”.

It is apparent that Engleman et al. did not appreciate the complexity of the methods necessary for inducing or assessing cytotoxic T lymphocyte killing activity using dendritic cells for antigen presentation. For example, it is apparent that they did not contemplate the need for use of an *immature* dendritic cell *lacking expression of the CD83 or DC-LAMP molecules* in order to optimize phagocytosis of antigen, which was presented in the context of an apoptotic cell. Nor did they anticipate the role for a *maturation factor* needed for subsequent *generation of a CD83 positive and DC-LAMP*

positive mature dendritic cell to optimize the induction and assessment of cytolytic T cells in response to antigen presentation by the dendritic cell.

With respect to the present invention, the Examiner's attention is drawn to page 71, lines 20-24 wherein it states:

*"Immature DCs efficiently phagocytose apoptotic cells. Based on previous observations that immature DCs are the cells responsible for capturing antigen (106), we predicted that apoptotic cells would be engulfed best by immature DCs."*

Furthermore, on page 73, lines 0-2, it states:

*"This data also demonstrates that it is the immature DC which preferentially acquires apoptotic material as compared to the mature DC."*

And yet further, on page 74, lines 9-14:

*"While mature DCs were efficient targets when infected with influenza, they were unable to cross-present antigens, presumably because they had down regulated the ability to phagocytose the apoptotic monocytes. The immature DCs, however, did cross-present antigens from apoptotic cells."*

And yet further on page 75, lines 10-26:

*"Immature DCs can be distinguished from macrophages by intracellular expression of CD83 and a unique profile of phagocytic receptors. We investigated the possibility that immature DCs might phagocytose apoptotic cells via pathways distinct from macrophages. To clearly distinguish these cells we characterized them phenotypically. Immature DCs are distinguished by the absence of both CD14, a macrophage restricted marker, and CD83, a maturation marker for DCs (125). We have extended the use of CD83, finding that immature DCs can be distinguished from both macrophages and mature DCs by their intracellular expression of CD83 [FIG. 18]. Macrophages do not express CD83 intracellularly nor extracellularly [FIG. 18], while mature DCs express CD83 both intracellularly and extracellularly [FIG. 18]."*

The role for a maturation stimulus to aid in the generation of CD83 and DC-LAMP positive mature dendritic cells, and their role in induction of cytolytic T cells can be found on page 91, lines 16-28:

*"Upon receipt of a maturation signal, DCs downregulate antigen acquisition, express higher levels of costimulatory and MHC molecules and become stably*

*differentiated to activate resting T cells. Maturation can be triggered by multiple stimuli including LPS, contact allergens, bacteria and viruses, cell products [monocyte conditioned medium-MCM, TNF $\alpha$ , IL-1 $\beta$ , PGE $_2$ , IFN-alpha (162-168)] and signalling molecules [CD 40L (169-170)]. Since it is the immature DC that captures antigen most efficiently, we investigated whether the uptake of dead or dying cells could initiate immunity by inducing DC maturation.”*

Further support for the role of mature dendritic cells in stimulation of T cells can be found on page 93, lines 30-33, continuing on to page 34, line 1, wherein it states:

*“Mature DCs are potent stimulators of T cells, the most straightforward assays being the induction of allogeneic T cell proliferation in the mixed leukocyte reaction [MLR] and superantigen dependent T cell proliferation (179, 180).”*

And finally on page 95, lines 15-32:

*“Phagocytosis of apoptotic cells by DCs failed to induce maturation, the consequences of which may be the induction of tolerance to self or tumor antigens (184-186). Phagocytosis of apoptotic cells, however, may lead to T cell immunity if followed by a maturation signal. We have shown that DCs phagocytose apoptotic cells and present antigens [e.g. viral and tumor antigens] from these sources to both CD4+ and CD8+ T cells. See, Examples 1-5. Given that phagocytosis of apoptotic cells does not mature DCs, signals provided by necrotic cells in the environment, such as cytokines [e.g. TNF $\alpha$ , IL-1 $\beta$ , IFN $\alpha$  released by virus infected cells], inflammatory products [e.g. LPS, bacterial cell walls] and CD4+ T cells [such as CD40-L/CD40 interactions (169, 170)] would be required to mature the DCs, thus allowing for the full activation of T cells.”*

Accordingly, Engleman et al. do not contemplate nor appreciate the need for antigen uptake in the context of an apoptotic cell by the “immature dendritic cell”, followed by subsequent maturation of the dendritic cell to induce or assess cytolytic T cell activity. It was only at the time of the present invention that such complexity became apparent with respect to optimization of these conditions.

Thus, Engleman et al. do not disclose nor suggest use of an immature dendritic cell lacking expression of surface CD83 and DC-LAMP and maintaining the CD83 cells as CD83 negative during the contacting of these cells with apoptotic cells expressing the antigen for antigen uptake. Nor do Engleman et al disclose or suggest use of mature,

surface CD83 positive and DC-LAMP positive dendritic cells for induction or assessment of cytolytic T cells.

In light of the foregoing claim amendments and arguments, Applicants respectfully request withdrawal of the rejection.

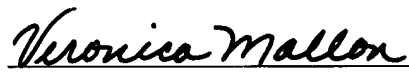
#### ***Fees***

No fees are believed to be due for the present response. However, should this be in error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment, or to credit any overpayments.

#### ***Conclusion***

Applicants believe that the foregoing amendments to the claims place the application in condition for allowance. Withdrawal of the rejections and objections is respectfully requested. If a discussion with the undersigned will be of assistance in resolving any remaining issues, the Examiner is invited to telephone the undersigned at (201) 487-5800, ext. 118, to effect a resolution.

Respectfully submitted,



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